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ECM between the control and the chitosan groups. Secondly, macrophages were stimulated with chitosan, and then transforming growth factor-beta 1 (TGF- β 1) and platelet-derived growth factor (PDGF) mRNA expressions and productions of their proteins were assayed *in vitro*. As a result, chitosan promoted the production of TGF- β 1 and PDGF. These results indicate that chitosan does not directly accelerate ECM production by fibroblast, and the ECM production may be increase by the growth factors.

The results of these studies showed the

mechanism of accelerated wound healing by chitosan as follows ; (1) Chitosan accelerates the migration of PMN at inflammation phase of healing. (2) Migrated PMN cells produce OPN which accelerates the migration macrophages. (3) The production of growth factor (TGF- β 1 and PDGF) is enhanced by chitosan stimulation. (4) These growth factors promote the proliferation of fibroblast and the production of ECM. (5) As a result, chitosan accelerates the granulation and wound healing.

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Apical delta formation of the root apex and efficacy of enamel matrix proteins in periodontal regeneration for periodontal disease in dogs

Kazuhiro Watanabe

*Laboratory of Veterinary Surgery
Department of Veterinary Clinical Sciences
Graduate School of Veterinary Medicine
Hokkaido University, Sapporo 060-0818, Japan*

Veterinary small animal dentistry has recently developed rapidly and is now respected as a veterinary medical discipline. Usually, the therapeutic techniques in human dentistry have been applied to small animal dentistry without any special considerations. However, various differences such as morphology, food, etc. between humans and animals are evident, and understanding these differences is essential to protect animal teeth from disease and to select the correct technique for treatment.

In dogs, apical lesions (apical abscesses) frequently form dental fistulae at ventral sites of the orbit and the root apex of

the canine eminence. The apical delta has a complex structure of the root apex in adult dogs, and this may be one of the factors causing the apical lesion of the tooth. The apical delta is an intricate system of cavities in which numerous passages of blood vessels and nerves branch from the pulp cavity to the root apex. This complicated structure makes complete removal of infected pulp by routine root canal treatment difficult. There are various reports on the rate of existence of the apical delta, but the root apexes immediately after the eruption of the permanent teeth in dogs are immature and have not yet closed. The eruption time also varies depending on

the kind of tooth. There is no report describing the relationship between the completion of the root apex and apical delta formation with aging of the dog.

Teeth with apical lesions in dogs are usually extracted. Apicoectomy is, however, able to conserve the tooth as a surgical endodontic therapy by the elimination of apical lesions. In this treatment, healing is considered to be repair, and regeneration can not be expected. Regeneration in dentistry means that the cementum consisting of the periodontal membrane and alveolar bone is newly re-formed. These attachment apparatuses achieve an important role in the conservation of teeth. It is considered that tissue healed by regeneration is more resistant to recurrence than tissue healed by repair.

On the other hand, there is a point of view that it is possible to mimic the phenomena that occur during root development to induce periodontal tissue regeneration in human periodontal disease. It has been reported that enamel matrix protein (EMP) applied onto the surface of the tooth root induces a series of phenomena leading to the regeneration of the adhesion system of the periodontal tissues and provides an environment for periodontal regeneration. There is, however, no clinical report on EMP application to dogs.

All permanent teeth root apices in growing beagle dogs and the permanent teeth extracted from adult dogs with periodontal diseases were examined histologically to determine the relationship of root apex closure and apical delta formation. In addition, the efficacy of the EMP used for human periodontal regeneration therapy was evaluated in experimental apicoectomy and spontaneous periodontal disease in dogs.

In the first study, the relationship of root apex structure and apical delta formation was studied. The dogs used for the experiments

were a litter of 7 beagles, and teeth were extracted from 6-, 7-, 8-, and 9-month-old dogs to gather all kinds of teeth. The central vertical roots were sectioned and stained with hematoxylin-eosin solution. Teeth was classified into the following root types; Type I (nonapical delta = nonapical closure), II (few apical delta), III A (low apical delta) and III B (high apical delta). In addition, the permanent teeth (208 teeth, 314 teeth roots) extracted from 33 adult dogs (4- to 15-year-old) of 12 breeds with periodontal disease at the Veterinary Teaching Hospital in Hokkaido University were investigated using ground sections. In the 6-month-old beagles, more than half (53%) were classified as Type I. In the 7-month-old dogs, Type III B was most predominant (76%) and Type I, II and III A were occasionally seen. In the 8- and 9-month-old animals, all root apices observed were Type III B. The majority of the teeth extracted from adult dogs with periodontal teeth were classified as Type III B (95%), and a few were Type III A. These results showed that the apical delta was formed quickly after apical closure, and already existed histologically in all beagles that were 8 months old. Mature apical deltas (Type III A and Type III B) also existed in all adult dogs of various breeds at 4 years of age and more. In humans, the apical ramification makes it difficult to treat teeth through the root canal, and dog teeth have a much more complicated structure of the apical delta. Thus, comprehension of this structure is necessary in canine root canal treatment.

In the second study, the efficacy of the EMP used for periodontal regeneration therapy after an experimental apicoectomy for assumed apical lesions of the teeth in dogs was histologically evaluated with regard to apical periodontal regeneration. The maxillary canine roots and maxillary fourth premolar buc-

cal mesial roots in five beagles (4 males and 1 female weighing 9-13kg and 14-31 months of age) were experimentally apicoectomized under general inhalation anesthesia. After the root apex was exposed and excised, the cementum was scraped off. EMP was injected into the defect space in the EMP group. In the control group, application of EMP was done alternately such that the tooth of one side was used as a control while its counterpart on the other side received EMP. After 12 weeks, dogs were euthanized, and the experimental teeth together with the surrounding soft and hard periodontal tissues were collected for histological evaluation under a light microscope. The following two points were evaluated; the size of the defect where the root apex was removed and the existence of the newly formed cementum and collagen fibers bridging the area between the new cementum and alveolar bone. As a result, in the EMP group, the size of the defect where the root apex was removed was smaller than that of the control group. The formation of new cementum was found in 8 of the 10 roots of the EMP group, and in 4 of the 10 roots of the control group. The newly formed collagen fibers bridged the area between the new cementum and the new alveolar bone in 7 of the 10 roots of the EMP group, but in none of those in the control group. The present results demonstrated marked apical periodontal regeneration after apicoectomy in

the EMP group. These results, therefore, suggest that the application of EMP can effectively induce the regeneration of periodontal structures in apicoectomized dogs.

In the third study, EMP was applied for regeneration of periodontal tissue in 2 dogs with spontaneous periodontal disease. Case 1 was a Shetland sheepdog that had bony resorption around the root and root apex of the maxillary right and left premolars, and case 2 was a beagle with vertical resorption of bone between the mandibular right first and second molars. A flap was made in the gingiva, and EMP was applied on the surface of the exposed root. As a result, an elevation of the gingiva and a gain of the clinical attachment level were observed, and a gain of the bone level was radiographically recognized. EMP was therefore suggested to be effective to induce dregeneration of periodontal tissues in cases with periodontal disease.

It is concluded that apical closure with formation of an apical delta already exist histologically in all dogs at 8 months of age. Therefore, comprehension of this structure is necessary in canine root canal treatment. The application of EMP after an apicoectomy for an apical lesions was useful for apical periodontal regeneration, and EMP was effective in the cases with spontaneous periodontal disease.